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Hypoglycemic Effects of Exo-biopolymers Produced by Five Different Medicinal Mushrooms in STZ-induced Diabetic Rats

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Hypoglycemic effects of exo-biopolymers (EBP) produced by submerged mycelial cultures of *Coriolus versicolor*, *Cordyceps sinensis*, *Paecilomyces japonica*, *Armillariella mellea*, and *Fomes fomentarius* were investigated in streptozotocin (STZ)-induced diabetic rats. The rats from each experimental group were orally administered with EBPs (100 mg/kg BW) daily for 2 weeks. Though the hypoglycemic effect was achieved in all the cases, however, *C. versicolor* EBP proved as the most potent one. The administration of the *C. versicolor* EBP substantially reduced (29.9%) the plasma glucose level as compared to the saline administered group (control). It also reduced the plasma total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST) and, alanine aminotransferase (ALT) levels by 9.22, 23.83, 16.93, and 27.31%, respectively. The sugar and amino acid compositions of this EBP were also analyzed in detail.

KEYWORDS : Exo-biopolymer, Hypoglycemic effect, Mushrooms

Recently, the use of dietary supplements, nutraceuticals, and functional foods have become a popular approach to prevent the occurrence of diabetes mellitus and to attenuate the complications of hyperglycemia in diabetic patients, which have potentially reduced the cost of the disease. Edible mushrooms have been recognized as the ideal food for dietetic prevention of hyperglycemia as they have high content of fiber, protein, and a low fat content (Zuomin *et al*., 1998). Many investigators have endeavored to study the hypoglycemic effect either from the fruiting body or mycelia of various edible/medicinal fungi including *Tremella aurantia*, *Cordyceps sinensis*, and *Fomes fomentarius*, though with little scientific evidence (Kiho *et al*., 1994, 1995; Lee, 2005). It has been reported that the fruiting bodies of *Cordyceps* sp., *Agrocybe cylindracea*, and *Auricularia auricula-judae* Que were found to be a potential functional food for diabetic patients (Kiho *et al*., 1996; Lo *et al*., 2004; Zuomin *et al*., 1998). However, most of the researches on the hypoglycemic effect were carried out either with the fruiting bodies or the mycelia of mushrooms. Nevertheless, some component, such as EBPs, when released into the culture medium during submerged fermentation of mushroom, documented to have various biological properties (Cavazzoni and Adami, 1992; Song *et al*., 1998). Recently, EBP have been investigated extensively as their production process from the culture broth do not require any extra steps and they also require a relatively simple purification process (Bae *et al*., 2000; Cavazzoni and Adami, 1992; Jong and Birmingham, 1992; Takasu *et al*., 1991). Therefore, it was worth to evaluate the hypoglycemic effect of EBPs produced by submerged mycelial culture of mushrooms. In the present investigation, the hypoglycemic activities of EBP produced by submerged mycelial culture of five different types of mushrooms have been studied in STZ-induced diabetic rats.

Materials and Methods

Strain. The various cultures of *Coriolus versicolor*, *Cordyceps sinensis*, *Paecilomyces japonica*, *Armillariella mellea*, and *Fomes fomentarius* were obtained from the Korean Agricultural Culture Collection, Suwon, Korea.

Production of exo-biopolymer. The seed culture was grown in 250 *ml* Erlenmeyer flask, containing 100 *ml* of potato dextrose broth medium (pH 4.5), and was incubated at 25°C/150 rpm/for 4 days. After 12 days of incubation, 100 *ml* of the medium with mycelial pellets were taken out and homogenized aseptically in a Sorvall omnimixer for 3 min in an ice bath and later inoculated (4% v/v) in the mushroom complete medium (MCM) of the following composition (g/*l*): glucose 20, yeast extract 2, peptone 2, KH_2PO_4 0.46, K_2HPO_4 1.0, and $MgSO_4 \cdot 7H_2O$ 0.5 with pH 5.0 for submerged fermentation. The submerged mycelial cultures were carried out in 500 *ml* Erlenmeyer flask, containing 250 *ml* of the MCM media at 25°C/150 rpm/for 9~12 days or in a 5-*l* jar fermentor (25°C/1.0 vvm/150 rpm). Culture broth was harvested by centrifugation (10,000 ×g/20 min) and the supernatant

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obtained was treated with ethanol. Ethanol precipitate was dissolved in water, dialyzed, and lyophilized to obtain as an EBP (Yang *et al.*, 2004).

Animals. Male Sprague-Dawley rats (130~150 g) obtained from the Daehan Biolink Co., Ltd. (Eumsung, Korea), were housed individually in stainless steel cages in a room with controlled temperature (22 ± 2°C), humidity (55 ± 5%) and with a 12 hr cycle of light and dark. The rats were fed with a commercial pelleted diet throughout the experimental period (Sam Yang Co., Korea).

Induction of diabetes and experimental design. The rats were acclimatized for 7 days in the growth chamber and then fasted for 12 hr before an intramuscular injection (50 mg/kg body weight) of STZ (Sigma) dissolved in a citrate buffer at pH 4.5 (Junod *et al.*, 1967). Two days after the treatment of STZ rats were considered to be diabetic as the non-fasting blood glucose concentrations were higher than 300 mg/d*l*. The diabetic state was further confirmed by the positive response of glucose in the urine (test strips; Glucotest, Germany). Thereafter, the animals were used as an insulin dependent diabetes mellitus (IDDM) models. After the induction of STZ diabetes, the rats were divided into six groups as follows: Control group, STZ diabetic rats administrated saline; EBPs group, diabetic rats administered exo-biopolymer extracts obtained from each mushroom (Table 1). The rats from respective group were administered with saline and EBP (100 mg/kg), using an oral zonde, daily for 2 weeks. The body weight gain and food intake were taken periodically.

At the end of oral administration, the animals were fasted for 9 hr and then immediately sacrificed following an abdominal incision under light ether anesthesia and then the blood was collected from the main artery. Blood samples were collected in heparinized tubes, and the plasma was separated by centrifugation (1,110 ×g/10 min).

Table 1. Experimental group for searching of hypoglycemic effect of the exo-biopolymers obtained from submerged culture of five different mushrooms

Group	Oral administration (100 mg/kg BW)***
Normal*	None
Control**	0.9% NaCl
CV**	Exo-biopolymer from *Coriolus versicolor.*
CS**	Exo-biopolymer from *Cordyceps sinensis.*
PJ**	Exo-biopolymer from *Paecilomyces japonica.*
AM**	Exo-biopolymer from *Armillariella mellea.*
FF**	Exo-biopolymer from *Fomes fomentarius*

*Normal rats (n = 6).
**Rats (n = 9) diabetes induced by streptozotocin (50 mg/kg BW).
***Rats from each experimental group were administered (100 mg/kg BW) orally, either with saline (control) or by different exo-biopolymers obtained from various mushrooms, daily for 2 weeks.

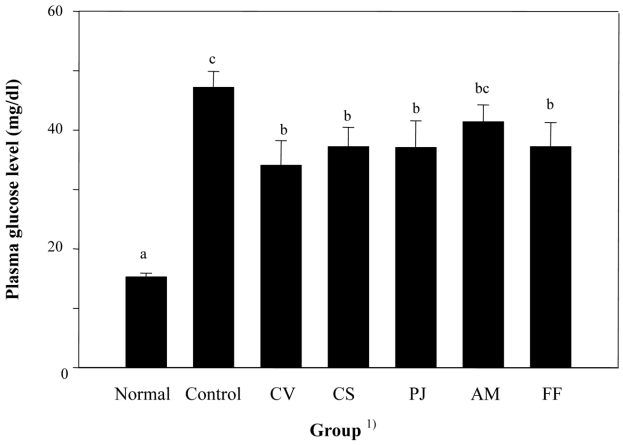

Fig. 1. Hypoglycemic effect of exo-biopolymers produced by submerged mycelial culture of different five mushrooms in streptozotocin-induced diabetic rats. [1)]See Table 1. Each value is the mean ± S.E. for 9 rats. [a,b,c]Values with different superscript letters in the same column significantly different among the group at $p < 0.05$.

The liver was perfused with cold saline, excised, weighed after washing with 0.9% NaCl, and then kept frozen (−70°C).

Biochemical analysis. The plasma glucose level was measured, using a glucose oxidase kit (glucose B-test, Wako Chemicals, Japan). The plasma TC, TG, ALT, and AST levels were evaluated by enzymatic test kits (Asan Pharm. Co., Korea). The liver TC and TG were assayed by the same method as described for the plasma TC and TG after the treatment with Triton X-100 (Preuss *et al.*, 1996).

Statistical analysis. Data were expressed as mean ± SE for 6 and 9 rats, accordingly. Group means were compared by one-way analysis of variance and by Duncan's multiple-range test (Duncan, 1957). The statistical differences were considered significant at $p < 0.05$.

Results and Discussion

Growth response. In the present investigation, the final body weight of diabetic group rats was found to be lower than that of the normal rat group, however, there was no significant difference between the control and the five EBP-administered groups (Table 2). The levels of food intake for the five EBP-administered groups were also not substantially different from that of the control group. These finding are in complementary with the result of Kim *et al.* (2001) who worked with five different mushrooms also reported that the level of food intake of five EBPs-administered groups were not substantially different from that the control group. Generally, the body

Table 2. Effect of exo-biopolymers produced through submerged mycelial cultures of five different mushrooms on the growth parameters of streptozotocin-induced diabetic rats for 2 weeks

Group[1)]	Body weight gain (g/day)	Food intake (g/day)	Food efficiency ratio[2)]
Normal	6.15 ± 0.26[b]	27.84 ± 1.39[a]	0.22 ± 0.11[b]
Control	3.71 ± 0.19[a]	40.59 ± 1.28[b]	0.09 ± 0.01[a]
CV	3.40 ± 0.41[a]	30.39 ± 1.82[a]	0.13 ± 0.02[a]
CS	4.10 ± 0.37[a]	39.57 ± 1.97[b]	0.11 ± 0.02[a]
PJ	3.74 ± 0.29[a]	37.90 ± 2.09[b]	0.12 ± 0.02[a]
AM	4.30 ± 0.33[a]	40.40 ± 1.80[b]	0.12 ± 0.01[a]
FF	4.10 ± 0.36[a]	39.76 ± 1.68[b]	0.11 ± 0.01[a]

[1)]See Table 1.
[2)]Body weight gain/Food intake.
Each value is the mean ± S.E. for 9 rats.
[a,b]Values with different superscript letters in the same column significantly different among the group at $p < 0.05$.

Table 3. Effect of exo-biopolymer produced through submerged mycelial culture of different mushrooms on plasma triglyceride and total cholesterol level in streptozotocin-induced diabetic rats for 2 weeks

Group[1)]	Triglyceride (mg/d*l*)	Total cholesterol (mg/d*l*)
Normal	31.38 ± 2.24[a]	71.80 ± 5.85[NS]
Control	51.70 ± 9.72[b]	78.95 ± 6.03
CV	43.00 ± 4.12[ab]	71.67 ± 2.15
CS	40.57 ± 1.72[ab]	73.23 ± 2.52
PJ	41.08 ± 3.82[ab]	78.83 ± 6.59
AM	50.04 ± 3.29[b]	78.71 ± 5.68
FF	42.94 ± 3.43[ab]	77.68 ± 7.18

[1)]See Table 1.
Each value is the mean ± S.E. for 9 rats.
[NS]Not significant.
[a,b]Values with different superscript letters in the same column significantly different among the group at $p < 0.05$.

weights were found to be reduced in STZ-induced diabetic rats and which can be recovered when subjected to the hypoglycemic treatment (Yang *et al*., 2004). The food efficiency ratio of rats was also found slightly reduced in the present studies. These results are complementary with the findings of other workers (Zuomin *et al*., 1998). The present results indicate that the body weight of all group animals was increased slightly. During investigation, the EBPs-administered groups caused no change in the gross behavior of experimental rats, thus leading to no death. Therefore, it could be said that there are no harmful effects in rats when EBPs were administered orally. Several workers have also reported similar results ruling out any possibility of harmful effects to the rats caused by oral administration of exo-polymer (Kim *et al*., 2001; Yang *et al*., 2004).

Hypoglycemic effects. The effect of EBPs of five different mushroom cultures on plasma glucose, TC and TG retention in diabetic rats were evaluated with respect to that of the saline-administered control group.

The plasma glucose level was significantly reduced after the oral administration of EBP obtained from *C. versicolor* (29.9%), followed by *P. japonica* (21.4%), *C. sinensis* (21.2%), *F. fomentarius* (21.2%), and *A. mellea* (12.3%), as compared to control group. The hypoglycemic effect exerted by the EBPs of these mushroom species in the present investigation may be due to its viscous nature. Such results confirm the findings of Kim *et al.* (2001). It has also been observed by several workers that a water-soluble dietary fiber (pectin and plant gum), showing high-viscosity, increases the gastric emptying time, as well as suppresses and/or delays the intestinal digestion and absorption of carbohydrates to prevent rapid blood glucose increase (Johnoson and Gee, 1981; Johnson, 1990; Jong and Birmingham, 1992). An increased viscosity of the intestinal content imposed by the EBP might have resulted in reduced nutrient movement towards the villi network for efficient absorption, which probably may play a role in lowering the level of plasma glucose (Kim *et al*., 2001). In fact, the findings of Gray and Flatt (1998) with a water-soluble extract of *Agaricus campestris* may possibly support this phenomenon. However, other mechanisms may also be involved in exhibiting the hypoglycemic effect by EBP. In this regard, the corrective role of EBP in pancreatic β-cells function cannot be ruled out as the STZ treatment inhibits the insulin secretion by the pancreas through selective destruction of β-cells of pancreatic islets (Mendola *et al*., 1989; Takasu *et al*., 1991; Yang *et al*., 2004). Hwang *et al*. (2005) suggested that the antihyperglycemic action might have been in part due to protection of β-cells against the cytotoxic action of STZ as evident by a retarted rate of insulin loss.

In the present investigation, except in AM group, the TG levels in plasma of all EBP administered groups, were obviously found to be lower than that of the control group (Table 3). The increased TG level in the diabetic rats may probably be associated with the low insulin level in the plasma (Schnatz *et al*., 1965). The significant TG lowering effect of *C. versicolor* (23.8%), followed by *C. sinensis* (21.5%), *P. japonica* (20.5%), *F. fomentarius* (16.9%) EBPs can be explained by their probable insulin-inducing activity, which promotes lipoprotein lipase (LPL) activity to reduce the plasma TG level (Kim *et al*., 2001). Increased mobilization of free fatty acids and decreased clearance due to reduced LPL activity results in elevated levels of TG and a very low density of lipoprotein (VLDL) in the blood plasma (Bar-On *et al*., 1976; Yang *et al*., 2004). The administration of EBP exhibited its effect on the plasma TC level slightly, which was also less significant. Plasma TG and TC levels are strongly related to the degree of diabetic control rats (Bar-On *et al*., 1976; Hayes, 1972; Yang *et al*., 2004). However, Koh and Choi

Table 4. Effect of exo-biopolymer produced through submerged mycelial culture of five different mushrooms on plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level in streptozotocin-induced diabetic rats for 2 weeks

Group[1)]	ALT (IU/*l*)	AST (IU/*l*)
Normal	10.07 ± 0.55[a]	46.36 ± 1.10[a]
Control	39.42 ± 6.25[b]	129.85 ± 15.59[b]
CV	32.75 ± 4.71[b]	94.39 ± 5.88[b]
CS	27.65 ± 3.99[b]	101.85 ± 10.79[b]
PJ	33.54 ± 4.59[b]	103.50 ± 12.06[b]
AM	39.05 ± 4.72[b]	117.74 ± 10.19[b]
FF	33.25 ± 4.03[b]	99.93 ± 15.53[b]

[1)]See Table 1.
Each value is the mean ± S.E. for 9 mice.
[a,b]Values with different superscript letters in the same column significantly different among the group at $p < 0.05$.

(2004) also reported that the concentration in plasma TC, LDL-cholesterol, and atherogenic index ratio was significantly low in the rats when fed with broth of mycelial culture of *C. versicolor*, which may be due to structural and compositional differences between culture broth and EBP obtained from the submerged mycelial culture.

In the present studies, ALT and AST level in plasma was significantly reduced by EBP of *C. versicolor* and *C. sinensis,* respectively, in STZ-induced diabetic rats (Table 4). Generally, ALT and AST levels are increased by metabolic change in the liver, such as administration of toxin, liver cirrhosis, hepatitis and liver cancer (Bursch and Schulte-Hermann, 1986). Perhaps the EBPs from *C. versicolor* and *C. sinensis* could enable the liver tissues to rectify the damaged by STZ administration, to some extent.

The present study has demonstrated the hypoglycemic potential of EBPs produced by submerged mycelial cultures of five different mushrooms in STZ-induced diabetic rats. The EBP obtained from *C. versicolor* was found to be the most potent one as compared to other. It has been observed that *C. versicolor* EBP administration attenuated the diabetogenic effect of STZ and which significantly reduced the degree of diabetes in the experimental organism. This result has encouraged us to believe that oral administration of *C. versicolor* EBPs may have a potential benefit in preventing diabetes, since pancreatic damage is induced by environmental chemicals (Lo *et al*., 2004). However, a further study is needed to identify the active fractions responsible for hypoglycemic activity and to clarify the mechanism of the effect. In future, combinations of other natural compounds may be envisaged to get more efficient hypoglycemic effect.

Acknowledegments

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